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CHANGES IN INTRACELLULAR FREE AMINO ACIDS (FAA) IN GILL, MANTLE AND ADDUCTOR MUSCLE TISSUE OF THE CAGED MUSSEL, ELLIPTIO COMPLANATA, EXPOSED TO CONTAMINATED ENVIRONMENTS

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ABSTRACT

Intracellular tissue concentrations of free amino acids (FAA) were monitored in caged mussels (Elliptio complanata) exposed in situ for 27-29 d and 77-79 d to various point and non-point sources of pollution in the Yamaska River watershed (Quebec, Canada). Total concentrations of FAA (nmol/mg wet weight) increased above background or control levels in both mantle and adductor muscle tissue at sites impacted by agricultural runoff and urban effluent from municipal sewage and light industries. These same sites were classified as poor to very poor in water quality using the Hilsenhoff biotic index for benthic invertebrate community health. Increases in total FAA levels could be attributed to increases in almost all individual FAA. Consistent changes in the % composition of individual FAA to the total FAA pool included decreases in serine, threonine, glycine and valine as well as increases in glutamic acid and glutamine at 27-29 d but results were not consistent with longer exposure and varied amongst sites. Few changes in total or individual FAA were observed in gill tissue. Condition indices (C.I.) were not correlated with changes in total FAA and decreased significantly only ($P<0.05$) at sites downstream of municipal sewage outfalls. The results from this and other studies suggest that increases in total FAA in some tissues of freshwater bivalves may be indicative of generalized stress induced by a variety of environmental factors and may be useful as an in situ biochemical index of toxicity.

RÉSUMÉ

Nous avons mesuré les concentrations intracellulaires en acides aminés libres ("free amino acids" ou FAA) dans les tissus de moules (Elliptio complanata) gardées en cage et exposées in situ, pour des périodes de 27-29 jours et 77-79 jours, à différentes sources ponctuelles et non ponctuelles de pollution dans le bassin de la rivière Yamaska (Québec, Canada). Les concentrations totales en FAA (nmol/mg de poids humide) dans le manteau et le muscle adducteur étaient supérieures à la concentration de fond ou à la concentration des témoins dans des endroits touchés par les eaux de ruissellement agricoles et par les eaux d'égout et les déversements de l'industrie légère des régions urbaines. La qualité de l'eau en ces endroits a été caractérisée comme étant pauvre à très pauvre selon l'indice biotique de Hilsenhoff servant à évaluer la santé des communautés d'invertébrés benthiques. L'augmentation des concentrations totales de FAA reflétait une augmentation de presque tous les FAA. Pour la période d'exposition de 27-29 jours, les variations de la composition en FAA étaient régulièrement attribuables principalement à une diminution des quantités de sérine, de thréonine, de glycine et de valine de même qu'à des augmentations des quantités d'acide glutamique et de glutamine, mais pour ce qui est de l'exposition de 77-79 jours, les résultats ne montraient pas la même régularité. De plus, ceux-ci variaient suivant les endroits. Dans les branchies, la quantité totale de FAA et celle de chacun des FAA ont été peu modifiées par

l'exposition à la pollution. Aucune corrélation n'a été observée entre les variations de la quantité totale de FAA et les indices de condition (C.I.). Ceux-ci ont montré une baisse significative ($P < 0,05$) seulement aux endroits situés en aval des émissaires d'évacuationmunicipaux. Les résultats de cette étude et d'autres études laissent entendre que les augmentations de la quantité totale de FAA dans certains tissus de bivalves peuvent servir d'indicateurs du stress global induit par différents facteurs environnementaux et peuvent donc s'avérer utiles comme indices biochimiques in situ de toxicité.

MANAGEMENT PERSPECTIVE

The detection of biochemical changes at the molecular and cellular level of organization in organisms exposed to chemical contaminants may be the earliest and most sensitive indication of a toxic response. These changes may in turn be indicative of decreases in the survival, growth and/or reproduction at the population and community level of organization. A number of biochemical indices to detect stress are being studied in the Contaminants Project, Rivers Research Branch, NWRI, for their potential use in the assessment of the ecological health of aquatic organisms exposed to toxicants from non-point source pollutants. One such biochemical tool is the measurement of changes in intracellular tissue concentrations of free amino acids (FAA) in molluscs. Total concentrations of FAA in marine organisms have been shown to decrease following exposure to toxic chemicals, but studies with freshwater organisms are rare. In the following paper, total FAA concentrations in various tissues of the caged mollusc, Elliptio complanata, were shown to increase after in situ long-term exposure in tributaries of the Yamaska River watershed, Quebec, Canada. These results are similar to those reported in other studies on freshwater organisms. Therefore, increases in total FAA concentrations in some tissues of freshwater molluscs may be a useful biomonitoring technique for future use by Environment Canada to assess in situ toxicity from non-point source pollutants.

PERSPECTIVE-GESTION

La détection de modifications biochimiques à l'échelle moléculaire et à l'échelle cellulaire chez les organismes exposés à des contaminants chimiques pourrait être l'indicateur le plus hâtif et le plus sensible d'une réaction à des produits toxiques. Les modifications observées peuvent à leur tour montrer que la survie, la croissance ou la reproduction d'une population ou d'une communauté sont menacées par des contaminants. Un certain nombre d'indices biochimiques permettant de détecter la présence de stress sont actuellement à l'étude dans le cadre du projet sur les contaminants de la Direction de la recherche sur les cours d'eau de l'Institut national de recherche sur les eaux afin d'examiner leur potentiel en ce qui a trait à l'évaluation de la santé écologique des organismes aquatiques exposés à des substances toxiques provenant de sources de pollution non ponctuelles. L'un de ces outils biochimiques consiste en la mesure des modifications des concentrations intracellulaires en acides aminés libres ("free amino acids" ou FAA) dans les tissus de mollusques. Il a été démontré que les concentrations totales en FAA chez les organismes marins diminuent suite à une exposition à des substances chimiques toxiques, mais les études portant sur les organismes d'eau douce sont rares. La présente étude montre que les concentrations totales en FAA mesurées dans différents tissus d'une espèce de mollusque, Elliptio complanata, gardé en cage augmentent après une exposition in situ à long terme dans des tributaires du

bassin de la rivière Yamaska, dans la province de Québec, au Canada. Les résultats de cette étude sont semblables à ceux d'autres études portant sur des organismes d'eau douce. Ainsi, les augmentations de la concentration totale en FAA dans certains tissus demollusques d'eau douce peut s'avérer une technique de bio-surveillance efficace que pourra utiliser Environnement Canada pour évaluer la toxicité in situ provenant de sources de pollution non ponctuelles.

INTRODUCTION

A basic premise of toxicology is that all toxic effects in living organisms begin with a reaction between the toxic chemical and some biochemical receptor (Dixon et al., 1985). Therefore, the earliest and most sensitive indication of a toxic response in organisms exposed to chemical contaminants should be measured by the detection of biochemical changes at the molecular and cellular level of organization (Graney and Giesy, 1988). In the aquatic environment, numerous biochemical indices of stress have been proposed for their potential use in the assessment of the "health" of populations, communities and ecosystems exposed to contaminants (Bayne et al., 1985). Various studies of marine invertebrates, particularly molluscs, have reported that total tissue concentrations of FAA either increase or decrease in response to natural and anthropogenic stresses such as changes in salinity (Wickes and Morgan, 1976; Baginski and Pierce, 1978; Bishop et al., 1981; McCoid et al., 1984; Matsushima, 1988), anoxia (Zurburg and Kluytmans, 1980; Powell et al., 1982), parasitism (Fent et al., 1970), starvation, temperature (Gabbott and Bayne, 1973) and exposure to toxic substances i.e., petroleum products, metals, PCBs, etc. (Jeffries, 1972; Roesijadi et al., 1976; Roesijadi and Anderson, 1979; Briggs, 1979; Carr and Linden, 1984; Kasschau and Howard, 1984).

In contrast to marine invertebrates, there have been relatively few studies on the patterns of FAA in the tissues of freshwater organisms exposed to stress. Gardner et al. (1981), compared the

composition and concentration of FAA in the mantle of the bivalve, Amblema plicata and reported elevated total FAA concentrations in organisms found in streams contaminated with acid coal mine drainage and trace metals when compared to organisms found in relatively unpolluted Missouri streams. Graney and Giesy (1988) found that short-term and long-term laboratory exposure of the freshwater clam, Corbicula fluminea to the anionic surfactant, sodium dodecyl sulfate, caused both an increase in total FAA concentrations and a change in the relative concentrations of individual amino acids. These authors suggest that alterations in FAA may be a more sensitive indicator of toxicant exposure than more traditional measures of effects (i.e., respiration) but caution that more research, especially in situ studies, are necessary before a decision can be made on the usefulness of FAA concentrations as a biochemical index of stress.

The objective of this research was to monitor the FAA pool of the freshwater mussel, Elliptio complanata, exposed in situ, in cages, to a variety of stressful environments in tributaries of the Yamaska River watershed, Quebec, Canada and to determine if changes occur in the FAA pool as a consequence of stress. The Yamaska River basin is known to be contaminated with a number of chemical pollutants including metals (Tessier et al., 1980; Croteau et al., 1984), nutrients from both municipal sewage outfalls and agricultural runoff (Campbell et al., 1976), pesticides (Muir et al., 1978) and various industrial pollutants (Auger et al., 1979).

MATERIAL AND METHODS

Study Area

The Yamaska River watershed (45°05' and 46°05'N latitude and 72°12' and 73°07'W longitude) is comprised of a number of tributaries draining a basin of approximately 4843 km^2 in southeastern Quebec towards Lake St. Peter, a widening of the St. Lawrence River between the cities of Montreal and Quebec. The location of each study site can be found in Figure 1 and Table 1. Table 1 also provides some pertinent information on the types of pollution prevalent upstream.

A number of physical and chemical parameters were measured at each of the study sites and are listed in Table 2. Major ions, nutrients and trace metals were determined from preserved and stored (4°C) water samples using standard methods (National Water Quality Laboratory, 1981).

Experimental Design

Specimens of the unionid mussel, Elliptio complanata, ranging in size from 7.0-8.5 cm valve length, were collected from a healthy

population in Balsam Lake (a pristine lake located in the Trent-Severn River system, southwestern Ontario, Canada) on June 4, 1987. The mussels were transported to the laboratory in Burlington, Ontario in coolers containing lake water (temperature 20°C), transferred to a fibreglass "Aquafarms" fish hatchery trough and held under a continuous flow of filtered dechlorinated water for 4 d. On June 8, 1987, mussels were transferred to a large cylindrical tank and transported to the study basin. No mortality was observed during this time period.

Fifteen mussels were place in each of two aluminum wire cages (25 x 25 x 11.5 cm) at approximately the same depth at each of the study sites. The cages were weighed down with bricks and attached by cables to iron reinforcing rods driven into the stream bed. Prior to placing the mussels in the cages at each site, each mussel was marked with a number using a utility knife and the valve length was measured to the nearest 1/10 mm using vernier calipers.

After 27-29 d and 77-79 d of exposure, cages containing mussels were retrieved at each site and 10 live mussels were removed from the cages (except where mortality or vandalism had reduced the number available), identified, measured, rinsed clean of mud, wrapped in pre-fired foil, and immediately frozen on dry ice. Mortality in each of the cages was noted and all dead animals were removed from the cages and discarded.

Collection of Benthic Invertebrate Samples

In addition to experiments with caged mussels, benthic invertebrate community assessments were conducted in both riffle and pool habitats at each study site using Surber and Hester-Dendy round multiple-plate samplers respectively. Three replicate surber samples (area 930 cm^2) were taken at each study site where shallow, fast-flowing riffle areas were present. The contents of each surber net were placed in 500 ml glass jars and preserved with 10% buffered formalin. The multiple-plate samplers were suspended approximately 25 cm from the stream bottom in pools similar to those where the caged mussels were placed and allowed to colonize for 7 weeks. Samplers were then retrieved, disassembled and all organisms and debris were scraped into a 200 µm sieve, rinsed and preserved with 10% buffered formalin.

In the laboratory, all benthic invertebrate samples were transferred to 70% ethanol, sorted, and identified to species wherever possible using the taxnomic guides of Wiggins (1977), Pennak (1978), Oliver and Roussel (1983) and Merritt and Cummins (1984). The Hilsenhoff biotic index (Hilsenhoff, 1987, 1988a) was calculated for each type of sample at each site. This index has been used successfully to assess the water quality in polluted and unpolluted streams in Wisconsin (Hilsenhoff, 1988b) and ranks sites on the basis of the response of the invertebrate community to organic pollution.

Biochemical Analysis

In the laboratory, three mussels from each site/exposure period were partially thawed and the adductor muscles (both posterior and anterior), mantle and gill tissues were dissected and weighed. Amino acids were extracted by homogenization of each tissue in methanol for 30 s, followed by centrifugation at 3000 rpm for 10 min. and filtration (0.45 Millex/HV) of the supernatant. Prior to extraction, 250 µl of a standard (norleucine) was added to each sample. The filtrates were stored in the freezer (-16°C) until analyses by high pressure liquid chromatography (HPLC).

Prior to analysis by HPLC, borate buffer (100 µl) was added to a subsample of each filtrate (400 µl) and the sample was derivatized with 500 µl of 9-fluorenylmethyl chloroformate (FMOC; Sigma Chemical Corp.) according to the method of Einarsson (1985). After 30 s reaction time, the excess reagent was removed by extraction with pentane.

The extracted samples were injected into a Waters HPLC system using a Varian fluorescence detector (λ_{ex} = 260 nm, λ_{em} = 310 nm) and reverse phase elution was employed using a C_{18} column. A constant flow rate of 1.7 mL/min was employed and the mobile phase varied from 25% acetonitrile in buffer to 50% ACN in buffer. Water used for the preparation of buffers and amino acid standards (Sigman Chemical Co.) was passed through a Millipore water purification system and Norganic cartridges (Waters Corp.). The FAA concentrations were normalized to wet weight (nmol/mg).

Condition Index

Condition indices were calculated for *E. complanata* collected on each sampling date and at each study site. The length of each mussel was measured to the nearest 0.1 mm using vernier calipers and the soft body tissues were removed and weighed. Wet weight was converted to dry weight using a conversion factor of 0.094 determined from *E. complanata* collected from the Ottawa River (J. Metcalfe, unpublished data). Condition indices (C.I.) were calculated using Equation 1 as follows:

Equation 1 $$C.I. = \frac{\text{dry weight (g)}}{\text{shell length (mm)}} \times 100$$

Statistical Analysis

Total FAA concentrations, percent contribution of specific amino acids to the total FAA pool and condition indices of mussels at sites exposed to pollution were compared to those of mussels from a control site using analysis of variance (ANOVA) followed by Dunnett's multiple range test ($P \leq 0.05$) (Steel and Torrie, 1985). All levels of significance are $P \leq 0.05$.

To determine if environmental variables (Table 2) could be correlated with the observed changes in total FAA, univariate linear regression analysis was performed for total FAA and each variable.

All statistical analyses were conducted on a microcomputer using the statistical package, SYSTAT Ver. 4.0 (Wilkinson, 1988).

RESULTS

Mortality of E. complanata

The percent mortality of caged E. complanata at most sites was very low (0% at most sites). Mortality was greatest at sites in the Riviere Yamaska nord (downstream from Granby; site #5) and Ruisseau Runnets, an agricultural site near Roxton Falls (site #31) after 27-29 d exposure (23% and 10% respectively). Few animals died between the first and second sampling dates. Unfortunately, observations on both sampling dates were complicated by the removal and scattering of animals from cages at sites #8 (27-29 d) and the control site (77-79 d) and the withdrawal of cages from the water at several agricultural sites (e.g., sites #30, #31 and #34) by vandals (77-79 d).

Changes in FAA Pool in Tissues of E. complanata

Adductor muscles

Alanine, glutamic acid, glycine and serine were found in the highest concentrations in adductor muscles (Tables 3 and 4) at most

sites on both sampling dates and contributed significantly to the total FAA pool i.e., 19.2%-32.8%, 6.2%-32.0%, 7.5%-13.7%, 6.7%-13% respectively. Other individual FAA found in substantial concentrations (5-10% of total FAA) were arginine, threonine, valine and isoleucine-leucine. Individual amino acids found in low concentration (<5.0% of total FAA) were asparagine, glutamine, proline, aspartic acid, methionine, and phenylalanine.

Concentrations of total FAA were increased significantly above control levels at Sites #4 and #33 after both 27-29 d and 77-79 d exposure and at sites #32 and #37 after 77-79 d exposure. Concentrations were also elevated at sites #5, #30, #31, #34, and #37 after 27-29 d, although these increases were not statistically significant. Total FAA concentrations were below those of the control at site #5 after 77-79 d exposure. No data are available for sites #30, #31 and #34 from the 77-79 d exposure period due to vandalism. The increases in total FAA concentrations at all sites could be attributed to increases in all individual amino acids with the exception of arginine at site #4 after 27-29 d exposure.

The % contributions of some individual FAA to the total FAA pool were significantly different at several sites. In general, these changes included decreases in serine, threonine, glycine and valine and increases in glutamine and glutamic acid at a number of sites (#5, #30, #31 and #33) but these changes were not consistent between sampling dates and some occurred at sites which did not show significant increases in total FAA.

Mantle

Individual FAA detected in the mantle of E. complanata were similar to those detected in the adductor muscles (Tables 5 and 6); however, the amino acids which were present in the highest concentrations differed. Alanine was again a dominant FAA, accounting for 17.7-27.4% of the total FAA pool but proline and valine were found in high concentrations and contributed 13.0-33.8% and 7.5-12.% respectively to the total FAA pool. Glutamic acid, glycine, and serine were less important and ranged from 4.0-9.8%, 5.4-7.8% and 5.4-7.2%, respectively. Percent contributions of other FAA were similar to those of adductor muscles.

Concentrations of total FAA were significantly increased at Sites #4, 30, 31, 33 and 34 after 27-29 d of exposure and at sites #4 and #32 after 77-79 d of exposure compared to animals at the control site. With the exception of Site #4 (downstream of Cowmansville), these sites again included those considered to be exposed to agricultural runoff. Total FAA were also elevated at Sites #8, 32 and 37 after 27-29 d and site #33 after 77-79 d exposure, but these increases were not statistically significant. Concentrations at sites #5 and #37 were below those of the control animals after 77-79 d of exposure. As with adductor muscles, no data for Sites #30, #31 and #34 after 77-79 d exposure were available due to vandalism.

Gill

Individual and total FAA concentrations and percent contributions to the entire FAA pool in gill tissue of E. complanata are presented in Tables 7 and 8. Of the 15 individual FAA detected, those contributing the most to the total FAA pool were alanine (14.6-19.8%) and glutamic acid (10.2-16.6%). Threonine, glycine, serine, proline, valine, and isoluecine-leucine were usually found contributing between 5-10% to the total FAA pool whereas arginine, glutamine, phenylalanine and methionine were found at lower concentrations (<5.0%).

Very few changes in either the total FAA pool or individual FAA concentrations were noted in animals exposed at the various sites. Total FAA levels increased significantly at Site #30 after 27-29 d and Site #32 after 77-79 d in situ. Increases in the % contribution of glutamine and glutamic acid and decreases in threonine occurred at Site #31 after 27-29 d exposure. Arginine increased and threonine and valine decreased at Site #33 after 77-79 d exposure.

Condition Index

Condition indices for E. complanata at all study sites were not significantly different after 27-29 d of exposure (Table 9). Exposure for 77-79 d resulted in a significant reduction in C.I. for animals at Site #8; C.I. was also lower but non-significant for animals downstream of Granby (Site #5).

Comparison of Sites Using the Hisenhoff Biotic Index

Values for the Hilsenhoff Biotic Index of community health based on species-level data are presented in Table 10 for both riffle (surber samples) and pool areas (artificial substrate samples) at each study site. As the Hisenhoff Index increases in value, it indicates a greater deterioration of the water quality as measured by the health of the benthic invertebrate community. The results indicated that the control site (Site #35) was reasonably clean and supported a healthy invertebrate community. Sites #4, #8 and #37 were moderately degraded and ranged from very good to fairly poor. The agricultural sites, Sites #30-33, as well as Site #5 ranked from poor to very poor, and were considered polluted. Site #34 was not assessed.

Statistical Correlations Between Environmental Variables and Changes in Total FAA

R^2 values for the univariate linear correlations between the environmental parameters measured at each of the study sites and the observed changes in total FAA for each of the three types of tissues are shown in Table 11. No environmental variable was significantly correlated with changes in total FAA for the types of tissues analyzed.

DISCUSSION

The transfer and introduction of mussels in cages from pristine environments to those polluted by toxic chemicals has been used as a successful biomonitoring tool in bioaccumulation studies for the past 15-20 years (Bedford et al., 1968; Curry, 1977/78; Kauss and Hamdy, 1985). E. complanata is ubiquitous in lakes and streams in northeastern Canada and the United States (Clarke, 1981), can live in a variety of environments with substrates ranging from mud and clay to sand and coarse gravel (Lewis and Riebel, 1984), and is therefore a good candidate for transferral experiments (Hinch and Green, in press). The present study is the first to measure changes in intracellular concentrations of FAA in caged freshwater mussels exposed to polluted environments.

Mortality and Condition Index

The mortality of caged E. complanata at Sites #5 and #31 after 27-29 d of exposure probably indicates an inability of susceptible animals to cope with stress at these two sites. Site #5 on the Rivière Yamaska Nord is located directly downstream from the industrialized town of Granby (population 46,652), and is in an area known to be impacted by municipal sewage treatment plant effluents and trace metals such as copper, zinc, lead, nickel and mercury (Auger et al., 1979; Tessier et al., 1980). In contrast, Site #31 is in a rural area

where pesticides and nutrients from corn production are known to contaminate the watershed (Muir et al., 1978) and water quality is considered to be poor (Auger et al., 1978). Agricultural practices are known to have a detrimental effect on aquatic invertebrates due to high bacterial and viral concentrations, high turbidity from erosion, and eutrophication from nutrient runoff (Dance and Hynes, 1980) and therefore a variety of factors could be contributing to mortality at this site.

Mortality of mussels at site #5 did not increase with a longer time of exposure (i.e., 77-79 d), although a decrease in C.I. at this site suggests that mussels were not as healthy as those exposed at other sites. C.I. is used as a general indicator of an animal's health and reflects the recent physiological history of the animal by measuring energy stored as glycogen, lipid and protein. It is related to the overall metabolic response of an animal to the conditions imposed by its environment and is known to decrease when animals are exposed to environmental stress such as overcrowding or starvation (Peddicord, 1977). However, C.I. may not always be a good indicator of deteriorating health. For example, Roesijadi and Anderson (1979) found that conditions indices in the clam, Macoma inquinata, exposed to oil-contaminated sediments were not significantly different from each other after 38 d even though 33% of the clams died in the treated sediments. C.I. was significantly reduced at site #8 after 77-79 d of exposure suggesting that the water quality at this site is also poor; however, the removal of animals from the cages and the scattering of

these organisms on the stream bed by vandals complicates the interpretation of results at this site.

Intracellular FAA Concentrations in *E. complanata*

The concentrations of total FAA in the various tissues of freshwater bivalves have been shown to range from 0.59-7.14 nmol/mg wet weight (this study; Gardner *et al.*, 1981; Graney and Giesy, 1988). These levels are substantially lower than those reported for marine and estuarine bivalves which range from 100-600 nmol/mg wet weight (Jeffries, 1972; Briggs, 1979; Roesijadi, 1979). As FAA are known to play a role in osmoregulation and the alteration of cellular volume in response to changes in salinity (Bayne *et al.*, 1985) and freshwater organisms are not exposed to great extremes of salinity; it is not surprising that these organisms have a lesser capacity for compensation and thus a smaller pool of FAA (Dietz, 1974; Hanson and Dietz, 1976).

In marine organisms, taurine, alanine, and glycine are the individual FAA which contribute most significantly to the total FAA pool (Zandee *et al.*, 1980). Taurine occurs less commonly in freshwater invertebrates and may in fact be absent (Bayne *et al.*, 1985). Gardner *et al.* (1981), detected a compound eluting at the retention time of taurine for the freshwater mussel, *Amblema plicata*, but its identify was never verified. Taurine was not detected in the present

study but alanine was consistently present in the greatest concentrations in all three types of tissue analyzed and contributed significantly to the total FAA pool. Glutamic acid was generally the second most prominent amino acid in adductor muscles and the gill whereas proline was found in greater abundance in the mantle. Valine and glycine were the third most predominant FAA in the mantle and adductor muscles respectively. Graney and Giesy (1988) found alanine, arginine and glutaminc acid to be the three most abundant amino acids in the adductor muscle of the freshwater clam, Corbicula fluminea. In the present study, arginine contributed approximately 5-10% to the total FAA pool in the adductor muscles and <5.0% in the mantle and gill. Studies with other freshwater mussels indicate that alanine, glutamic acid and glycine are predominant in the mantle (Hanson & Dietz, 1976; Gardner et al., 1981; Graney and Giesy, 1988).

A comparison of all data for total FAA in all three tissue types (Table 12) suggests that, in general, total concentrations of FAA became elevated at a number of sites exposed to various sources of contamination although not all increases were statistically significant. Significant increases in total FAA concentrations in both adductor muscles and mantle occurred at sites #4 and #33 after 27-29 d exposure and 77-79 d exposure for mantle tissue. Increases in total FAA at sites #30, #31, and #34 also occurred in mantle (significant), adductor (n.s.) and gill (#30 only) after 27-29 d exposure; no data were available to determine if these increases persisted following longer exposure due to vandalism. Total FAA levels were also elevated at site #37 (n.s.) after 27-29 d and 77-79 d (significant) in adductor

muscle and after 27-29 d in mantle (n.s.). Site #32 showed significant increases in total FAA levels only after 77-79 d exposure but these increases occurred in all three tissue types.

Several other studies with freshwater bivalves have reported increases in total concentrations of intracellular FAA in organisms exposed to toxicants in both the laboratory and the field. For example, Gardner et al. (1981), found that total FAA levels in the mantle of natural populations of native Amblema plicata were higher in mussels from streams with a history of exposure to acid coal mine drainage and trace metals than those organisms collected from relatively unpolluted streams. In laboratory studies, Graney and Giesy (1988) showed that acute and chronic exposure of the clam, Corbicula fluminea, to sodium dodecyl sulfate caused increases in the total FAA concentrations in both adductor muscle and mantle tissue. These results are in contrast with marine bivalve studies in which animals exposed to a variety of natural and manmade stresses, i.e., oil-contaminated sediments (Roesijadi and Anderson, 1979); drilling effluents, anoxia, turbidity (Powell et al., 1982); cadmium (Briggs, 1979) and various salinities (Baginski et al., 1978) had lower intracellular tissue concentrations of FAA than those of the controls.

Concentrations of total FAA did decrease at several sites and sampling dates in the present study. For example, total FAA levels decreased in the mantle at sites #5 and #37 after 77-79 d exposure and #5 after 27-29 d and 77-79 d for adductor muscle. Other studies with freshwater invertebrates (e.g., the amphipod, Gammarus pseudolimnaeus) have also observed a decrease in total FAA concentrations following

short-term exposure to toxicants (Graney and Giesy, 1986; Graney and Giesy, 1987). Whether a specific response to a toxicant is an increase or decrease in total intracellular FAA concentrations may be dependent upon the type of contaminant present, the organism affected and the specificity of the mode(s) of action of the contaminant (see below).

Changes in the % contributions of certain individual free amino acids to the total FAA pool have also been reported as indicators of a response to stress. In marine organisms exposed to contamination, an increase in the tissue molar ratio of taurine:glycine is often observed due to a decrease in the concentration of glycine (Jeffries, 1972; Bayne et al., 1985). A decrease in the sum of the individual amino acids, serine and threonine, is also thought to indicate a response to stress (Bayne et al., 1985). Decreases in the % contributions of glycine, serine and threonine as well as valine to the total FAA pool in the present study occurred at site #5 and several of the agricultural sites (#30, #31 and #33) and may be indicative of a response by E. complanata to stress. Significant increases in the % composition of glutamine and glutamic acid were also observed in adductor muscle tissue at these same sites. Increases in glutamic acid levels have also been noted as a consistent response to trace metal pollution in sea anenomes, Bunodosoma cavernata (Kasschau et al., 1980) and in the adductor muscle of the grass shrimp, Palaemonetes pugio, following exposure to PCB's (Roesijadi et al., 1976).

It is possible, albeit subjective, to arrange the sites in the present study from the most to the least stressful based on observed mortalities and changes in total and individual FAA levels as shown in Table 13.

Comparison of these results to the ranking of sites as predicted by the Hilsenhoff Biotic Index indicates some similarities, i.e., the agricultural sites (sites #30-34) and site #5 appear to be the most deteriorated compared to the control site. Evidence in support of toxicity at these sites is given in Bird (1989) in a study of the incidence of mouthpart deformities in populations of chironomids at all sites in the Yamaska River watershed. he found that the freuqency of deformities (>1%) at sites #5, #30, #32 and #33 indicated problems with water pollution and suggested that agricultural pesticides, which are known to be prevalent at Sites #30, #32 and #33 (Muir et al., 1981), are impacting the benthic fauna at these sites. Deformities at Site #5 were attributed to exposure to trace metals accumulated in the sediment from several metal processing plants in Granby (Tessier et al., 1980).

In the present study, site #4 is classified as very contaminated on the basis of significant increases in total FAA, but the B.I. indicates only moderate deterioration in water quality. The discrepancies in these data may be explained by the fact that B.I. are

designed to rank degradation due to organic pollution whereas changes in total FAA levels are more indicative of toxicity at the molecular level due to specific toxicants.

There was very little correlation between the observed changes in total FAA and the water quality parameters measured in this study. These data indicate that the water quality parameters measured are not individually responsible for the observed biotic responses. Although environmental conditions such as low dissolved oxygen or high temperatures can have a detrimental effect on aquatic organisms (Gabbott and Bayne, 1973; McMahon, 1979), extremes in these parameters and others (i.e., pH, depth, velocity) were not noted. Levels of nutrients (e.g., N, P) ranged from 0.22-5.68 mg/L for NO_3NO_2 and 0.053-0.536 mg/L for phosphorus respectively, and were higher in the agricultural areas but did not appear to be specifically contributing to toxicity. Concentrations of several metals, most notably aluminum, iron, cobalt and zinc, were above the Canadian Water Quality Guidelines (CRREM, 1987) at several of the agricultural sites where total FAA levels were also elevated but statistical correlations were not significant. Interactive effects of two or more parameters could be contributing to the observed response but were not specifically studied.

Mechanisms of Action for Changes in FAA in Response to Stress

The biochemical regulation of intracellular FAA pools in aquatic invertebrates is poorly understood (Gainey, 1978). In addition, there is considerable variation in the magnitude and type of FAA changes which can occur in freshwater and marine organisms exposed to stress (Bishop et al., 1983). Nevertheless, three potential mechanisms for changes in the concentrations of FAA in stressed invertebrates have been suggested (ca. Powell et al., 1982; Graney and Geisy, 1988).

Many invertebrate species are known to utilize intracellular FAA as osmotic effectors in the regulation of cell volume during salinity stress (Sansone et al., 1978). Concentrations of total FAA have been observed to decrease when exposed to hypoosmotic conditions and increase under hyperosmotic conditions (Bayne et al., 1985). The observed increases in total FAA concentrations in the present study were not likely in response to increases in salinities in the freshwaters of the Yamaska River watershed although salinities were not measured in this study. Conductivity, which is a measurement of the ionic strength of freshwater, was taken at each site and ranged from 136-630 µmhos/cm. There was no significant correlation between total FAA concentrations and conductivity. The concentrations of solutes in

the hemolymph and tissues of freshwater molluscs are much greater than the environment in which they live and therefore small increases in ionic composition would have little effect on total FAA concentrations.

It is likely that unique metabolic pathways are present in invertebrates to respond to specific intracellular needs brought on by changes in salinity. Certain toxicants may impair these complex mechanisms but the consequence of such impairment should result in changes in only a few individual FAA (i.e., glycine, alanine, etc.) (Powell et al., 1982) rather than the entire FAA pool. Increases in almost all individual FAA contributed to the total increases in the FAA pools in the present study and this indicates that a more general response to stress rather than a specific alteration caused by a unique mechanisms of toxicity is occurring. In addition, it is unlikely that toxicants which act specifically on the processes and pathways of osmoregulation would be present at all sites in the Yamaska River watershed where inputs of toxicants varied.

Alterations in intracellular concentrations of FAA have also been attributed to anoxic conditions either in the external medium to which the animal is exposed (Dietz, 1974) or to self-induced anoxia by the closure of the valves to avoid toxic conditions (Farris et al., 1988). Dissolved oxygen (mg/L) at the study sites in the present experiment ranged from 6.4-10.8 mg/L; therefore, anoxic conditions did not occur in the external medium. Specimens of E. complanata were not observed in situ so it is possible that valve closure could have

occurred for extended periods of time in response to toxic conditions at each site. However, other studies on changes in concentrations of FAA in molluscs exposed to anoxic conditions have observed changes in only certain individual FAA in the total pool rather than a general increase in all FAA levels. For example, Zandee et al. (1980) and Powell et al. (1982) reported that exposure of the mussel, Mytilus edulis and the oyster, Crassostrea virginica, to anoxic conditions resulted in increases in alanine and glutamic acid levels and decreases in aspartic acid.

A general increase in many individual FAA resulting in an overall increase in total FAA concentrations can be explained by two theories (Bishop, 1983). Most non-essential amino acids (e.g., alanine, glycine, proline, asparagine, etc.) can be synthesized in invertebrates by the transammination of glutamic acid with an appropriate Krebs cycle intermediate. Glutamic acid is synthesized from NH_4^+ and α-ketoglutarate by the action of the enzyme glutamate dehydrogenase (GDH). Therefore, increases in FAA levels could be accounted for by increases in GDH activity which favours increased production of glutamaic acid thus giving rise to other amino acids. Wickes and Morgan (1976) found that levels of GDH were very low in the gill but high in the mantle and adductor muscle of the oyster, Crassostrea virginica. Therefore, stimulation of GDH in the mantle and adductor tissue of E. complanata but not the gill could explain why relatively few changes in total or individual FAA were noted in the gill but were apparent in adductor muscle and mantle. However, GDH does not play a

role in the synthesis of several non-essential FAA (e.g., proline) as well as the essential FAA such as valine, leucine, isoleucine, etc., which must be obtained in the diet (Stryer, 1981). Several of these amino acids also increased in the present study and these increases are difficult to explain simply on the basis of stimulation of GDH. In addition, there is no scientific evidence to show that stimulation of GDH by toxicants occurs in molluscs.

The accumulation of many FAA in intracellular tissue can be better explained by proteolysis which causes the release of amino acids from proteins. For many invertebrates, the amino acids generated by protein degradation can contribute significantly to the total energy budget of the organisms. The mobilization of energy reserves in aquatic invertebrates under stress has been observed (Riley, 1980; Riley and Mix, 1981; Carr and Linden, 1984) and is thought to be necessary for the maintenance of homeostasis. This type of stress response would result in increases in the entire FAA pool with individual FAA being affected more or less equally so that the proportions present within the FAA pool shift relatively little. These types of changes in FAA levels were observed in the present study although the relative percent contributions of several amino acids also changed at some study sites.

The mobilization of energy reserves by E. complanata should be reflected in a decrease in C.I. which measures levels on glycogen, lipids and proteins in bivalves. This was not observed in this study; in fact, C.I. was decreased only at the two sites where total FAA concentrations were at or below the levels of the control.

The FAA pool of invertebrates is a centre of metabolic activity with many complex multicomponent pathways for the synthesis and release of amino acids. Powell *et al*. (1982) suggests that two stresses presented concurrently might superimpose two distinct metabolic phenomena which modify each other to some extent resulting in a FAA pattern different from that produced by either one alone, i.e., some additive or synergistic effects may be found. This could explain why the C.I. in this study indicates a deterioration in the health of organisms without a concurrent increase or decrease in concentrations of FAA at sites with a variety of chemical inputs.

The results of this study do not prove that the changes in FAA in molluscs were induced as suggested above. However, the evidence from this and other studies supports the basic concept that exposure of invertebrates, particular molluscs, to stressful environments results in either increases (generally in freshwater organisms) or decreases (generally in marine invertebrates) in the entire FAA pool or specific components within that pool depending on species, environmental conditions and the severity and type of stress.

Further research in this area is essential before monitoring changes in concentrations of total or individual FAA can become a viable *in situ* ecotoxicological tool.

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Table 1. Type and degree of pollutant upstream of study site.

Site	Location	Population Upstream	Sewage Input	% Land Under Agriculture	Industry
35	Ruisseau Runnets	<1,000	light	11	nil
4	Rivière Yamaska Sud-est	10,000	moderate-heavy	6.5	moderate
5	Rivière Yamaska Nord	>40,000	heavy	5	heavy
8	Rivière Noire	2,500	light	3	light
30	Rivière a là Barbue	<1,000	light	45	light-moderate
31	Ruisseau Runnets	<1,000	light	14	nil
32	Rivière Saint-Nazaire	<1,000	light	39	light
33	Rivière Chibouet	<1,000	light	55	light
34	Rivière Salvail	<1,000	light	N.A.	light
37	Rivière Yamaska Nord	4,500	light-moderate	5	moderate

N.A. - not available

Table 2. Environmental variables measured at each site in the Yamaska River watershed.

Depth of water at cage (cm)	Nutrients (mg/L)
Velocity (cm/sec)	
Temperature (°C)	NH_3N
Suspended sediment (mg/L)	NO_3NO_2
	Total Phosphorus (µg/L)
Dissolved oxygen (DO) (mg/L)	
pH	
Alkalinity $CaCO_3$ (mg/L)	Metals
Conductivity (µmhos/cm)	
	Aluminum
Particulate organic carbon (POC) mg/L	Cadmium
	Cobalt
Dissolved organic carbon (DOC)	Copper
Dissolved inorganic carbon (DIC)	Iron
	Manganese
Major ions (mg/l)	Nickel
Calcium	Lead
Magnesium	Zinc
Sodium	
Chloride	

Table 3. Concentration (nmol/mg wet weight) and percent composition of FAA in adductor muscles of E. corplanata after in situ exposure for 27-29 d.

Amine Acid	35 (Control)	4	5	8	30	31	32	33	34	37
Alanine	0.56[a] (0.14) 23.2 [b] (3.3)	0.84 (0.21) 21.8 (3.8)	0.44 (0.04) 19.2 (1.2)	0.50 (0.14) 25.1 (3.4)	0.69 (0.04) 22.8 (4.3)	0.74 (0.10) 22.0 (1.1)	0.49 (0.12) 22.4 (4.3)	0.97 (0.24) 25.0 (7.0)	0.88 (0.17) 26.8 (3.1)	0.84 (0.23) 22.7 (1.1)
Glutamic Acid	0.16 (0.06) 6.7 (1.5)	0.31 (0.08) 8.1 (0.3)	0.75 (0.06) 32.0[e] (2.1)	0.16 (0.02) 8.5 (2.0)	0.41 (0.09) 13.0[e] (1.2)	0.46 (0.13) 13.8[e] (4.6)	0.27 (0.05) 12.4[e] (0.5)	0.47 (0.06) 11.8[e] (1.1)	0.26 (0.03) 7.9 (1.0)	0.34 (0.09) 9.1 (2.0)
Glycine	0.29 (0.03) 12.5 (3.2)	0.42 (0.12) 10.8 (1.9)	0.19 (0.02) 7.5[d] (0.7)	0.28 (0.10) 13.7 (1.4)	0.25 (0.05) 8.0[d] (0.6)	0.28 (0.09) 8.3[d] (2.0)	0.22 (0.06) 9.8 (1.7)	0.31 (0.05) 7.7[d] (1.3)	0.34 (0.03) 10.6 (2.1)	0.43 (0.12) 11.7 (0.5)
Serine	0.27 (0.04) 11.3 (1.4)	0.43 (0.12) 11.0 (0.6)	0.17 (0.06) 6.7[d] (1.8)	0.26 (0.10) 13.0 (1.0)	0.2[b] (0.08) 8.3[d] (1.4)	0.25 (0.06) 7.3[d] (1.2)	0.20 (0.08) 9.0 (2.0)	0.33 (0.07) 8.3[d] (1.6)	0.30 (0.02) 9.1 (0.3)	0.40 (0.11) 10.8 (1.4)
Arginine	0.26 (0.17) 10.1 (5.1)	0.21 (0.12) 5.2 (2.2)	0.17 (0.06) 6.5 (2.1)	0.09 (0.03) 4.6 (0.2)	0.22 (0.02) 7.3 (0.5)	0.23 (0.06) 7.0 (2.0)	0.22 (0.10) 10.7 (5.9)	0.33 (0.13) 8.2 (3.1)	0.24 (0.06) 7.2 (1.2)	0.21 (0.05) 5.8 (2.2)
Threonine	0.19 (0.02) 8.1 (1.4)	0.29 (0.05) 7.7 (1.0)	0.12 (0.02) 4.9[d] (0.5)	0.18 (0.04) 9.2 (0.8)	0.22 (0.03) 7.2 (0.6)	0.16 (0.02) 4.8[d] (0.6)	0.17 (0.06) 7.6 (1.3)	0.31 (0.03) 7.8 (0.6)	0.25 (0.03) 7.7 (1.0)	0.28 (0.08) 7.5 (0.9)
Valine	0.15 (0.02) 6.1 (1.1)	0.25 (0.06) 6.4 (0.8)	0.10 (0.02) 4.1 (0.5)	0.13 (0.04) 6.7 (0.5)	0.17 (0.04) 5.3 (0.4)	0.12 (0.02) 3.6[d] (0.5)	0.12 (0.04) 5.3 (0.9)	0.18 (0.02) 4.5 (0.5)	0.18 (0.02) 5.5 (0.6)	0.24 (0.07) 6.3 (0.8)
Glutamine	0.07 (0.01) 2.8 (0.2)	0.17 (0.08) 4.0 (1.1)	0.05 (0.01) 1.9[d] (0.3)	0.04 (0.03) 2.1 (1.6)	0.18 (0.05) 5.6[e] (0.8)	0.63 (0.16) 18.9[e] (5.0)	0.06 (0.01) 2.7 (0.3)	0.28 (0.05) 7.2[e] (1.3)	0.12 (0.03) 3.4 (0.4)	0.16 (0.04) 4.4 (0.9)
Proline	0.10 (0.02) 4.1 (0.2)	0.18 (0.03) 4.6 (0.9)	0.07 (0.01) 4.4[d] (2.4)	0.09 (0.02) 4.7 (0.6)	0.14 (0.02) 4.6 (1.3)	0.10 (0.05) 3.0 (1.2)	0.08 (0.03) 3.4 (1.0)	0.21 (0.11) 5.4[d] (3.1)	0.23 (0.07) 7.1 (2.0)	0.17 (0.06) 4.5 (0.6)
Isoleucine/ Leucine	0.20 (0.05) 7.5 (0.8)	0.50 (0.16) 12.6[e] (1.5)	0.20 (0.04) 8.0 (1.3)	0.19 (0.13) 8.6 (3.5)	0.32 (0.09) 10.1 (1.3)	0.20 (0.06) 5.2 (1.3)	0.23 (0.08) 10.1 (2.0)	0.30 (0.10) 7.2 (2.5)	0.27 (0.02) 8.1 (1.5)	0.41 (0.12) 11.1 (1.1)
Others[c]	0.16 (0.02) 7 (0.1)	0.30 (0.16) 7.4 (2.5)	0.14 (0.05) 4.6 (0.6)	0.05 (0.03) 2.1 (1.0)	0.25 (0.08) 8.1 (1.9)	0.18 (0.06) 5.2 (1.3)	0.15 (0.02) 6.9 (0.9)	0.28 (0.02) 7.1 (0.5)	0.22 (0.08) 11.5 (5.4)	0.26 (0.10) 7.0 (2.6)
Totals	2.42 (0.39)	3.89[e](1.00)	3.30 (1.64)	2.00 (0.64)	3.10 (0.48)	3.36 (0.26)	2.21 (0.39)	3.97[e](0.10)	3.29 (0.35)	3.70 (0.93)

[a] concentration (nmol/mg wet weight) (S.D. in parentheses; n = 3)
[b] percent contribution to total FAA pool
[c] includes aspartic acid, asparagine, methionine and phenylalanine
[d] significantly decreased from control ($P \leq 0.05$)
[e] significantly increased from control ($P \geq 0.05$)

Table 4. Concentration (nmol/mg wet weight) and percent composition of FAA in adductor muscles of *E. complanata* after *in situ* exposure for 77-79 d.

Amine Acid	35	4	5	32	33	37
Alanine	0.52[a] (0.03) 27.8[b] (3.2)	0.87 (0.08) 24.9 (0.4)	0.43 (0.19) 25.1 (3.8)	1.56 (0.29) 21.8 (1.8)	0.91 (0.22) 24.3 (1.0)	0.92 (0.07) 25.1 (1.8)
Glutamic Acid	0.16 (0.03) 8.4 (1.0)	0.27 (0.02) 7.7 (1.1)	0.17 (0.05) 10.0 (2.0)	0.55 (0.09) 7.6 (0.5)	0.30 (0.09) 8.0 (1.1)	0.29 (0.20) 7.9 (1.2)
Glycine	0.20 (0.05) 10.4 (2.0)	0.43 (0.04) 12.3 (2.2)	0.20 (0.08) 12.3 (1.9)	0.74 (0.09) 10.4 (1.4)	0.42 (0.10) 11.2 (0.3)	0.42 (0.05) 11.3 (0.4)
Serine	0.17 (0.03) 9.1 (1.5)	0.33 (0.02) 9.6 (1.0)	0.17 (0.05) 10.4 (2.1)	0.72 (0.09) 10.0 (0.4)	0.37 (0.08) 10.0 (2.0)	0.36 (0.06) 9.8 (2.4)
Arginine	0.11 (0.02) 5.7 (1.3)	0.23 (0.18) 6.2 (4.2)	0.03 (0.02) 1.4[d] (0.9)	0.34 (0.06) 4.8 (1.1)	0.17 (0.04) 4.4 (0.2)	0.13 (0.03) 3.7 (0.8)
Threonine	0.16 (0.02) 8.3 (1.2)	0.26 (0.03) 7.5 (0.8)	0.15 (0.05) 9.6 (0.9)	0.50 (0.06) 7.0 (0.2)	0.27 (0.07) 7.1 (0.4)	0.26 (0.03) 7.1 (0.4)
Valine	0.10 (0.01) 5.4 (0.5)	0.20 (0.01) 5.7 (0.4)	0.11 (0.03) 6.4 (1.0)	0.47 (0.07) 6.6 (0.1)	0.22 (0.06) 5.8 (0.5)	0.24 (0.04) 6.4 (0.5)
Glutamine	0.05 (0.01) 2.6 (0.2)	0.11 (0.03) 3.1 (0.7)	0.04 (0.01) 2.5 (0.6)	0.27 (0.04) 3.8 (0.3)	0.17 (0.09) 4.3 (1.5)	0.16 (0.06) 4.2 (1.3)
Proline	0.16 (0.08) 8.3 (4.0)	0.19 (0.09) 5.4[d] (0.6)	0.07 (0.03) 4.4[d] (0.7)	0.42 (0.07) 5.9 (0.4)	0.22 (0.06) 5.9 (0.2)	0.20 (0.04) 5.4 (0.8)
Isoleucine-Leucine	0.16 (0.04) 8.4 (1.7)	0.38 (0.07) 10.7 (1.9)	0.13 (0.05) 7.7 (1.2)	1.00 (0.14) 13.9[e] (0.3)	0.44 (0.18) 11.4 (2.3)	0.41 (0.07) 11.0 (1.8)
Others[c]	0.14 (0.04) 7.4 (2.0)	0.24 (0.05) 6.8 (1.0)	0.04 (0.02) 2.6[d] (0.6)	0.58 (0.15) 8.5 (1.1)	0.27 (0.08) 8.5 (2.4)	0.31 (0.11) 8.3 (2.4)
Totals	1.89 (0.16)	3.49[e] (0.36)	1.54 (0.56)	7.15[e] (0.95)	3.76[e] (0.97)	3.68[e] (0.33)

[a]concentration (nmol/mg wet weight) (S.D. in parentheses; n = 3)
[b]percent contribution to total FAA pool
[c]includes aspartic acid, asparagine, methionine and phenylalanine
[d]significantly decreased from control (P = 0.05)
[e]significantly increased from control (P = 0.05)

Table 5. Concentration (nmol/mg wet weight) and percent composition of FAA in adductor muscles of *E. complanata* after *in situ* exposure for 27-29 d.

Amine Acid	35 (Control)	4	5	8	30	31	32	33	34	37
Alanine	0.24 (0.01) 23.6 (3.5)	0.49 (0.09) 24.4 (1.1)	0.33 (0.12) 27.4 (3.2)	0.32 (0.22) 19.1 (6.4)	0.60 (0.16) 22.0 (0.4)	0.50 (0.19) 24.4 (3.5)	0.44 (0.10) 22.6 (0.8)	0.44 (0.12) 22.7 (3.3)	0.47 (0.05) 23.5 (1.0)	0.30 (0.03) 21.6 (2.1)
Proline	0.21 (0.09) 19.6 (3.6)	0.31 (0.08) 15.4 (1.7)	0.18 (0.03) 15.3 (2.1)	0.49 (0.26) 33.8[e] (23.5)	0.48 (0.15) 17.7 (1.6)	0.35 (0.07) 17.6 (2.2)	0.30 (0.02) 15.8 (3.0)	0.25 (0.06) 13.0 (2.3)	0.30 (0.05) 15.0 (2.6)	0.36 (0.07) 25.8 (4.0)
Valine	0.13 (0.03) 12.5f (0.5)	0.22 (0.02) 10.9 (1.1)	0.12 (0.03) 10.1[d] (0.6)	0.14 (0.06) 8.6[d] (1.3)	0.28 (0.05) 10.4 (1.1)	0.15 (0.05) 7.5[d] (0.3)	0.24 (0.08) 11.9 (1.5)	0.19 (0.04) 1.0[d] (2.7)	0.21 (0.03) 10.4 (1.3)	0.16 (0.02) 11.5 (0.3)
Threonine	0.10 (0.03) 9.6 (0.9)	0.18 (0.05) 8.8 (0.9)	0.10 (0.03) 8.3 (0.4)	0.13 (0.09) 7.7 (3.4)	0.24 (0.06) 8.7 (1.2)	0.14 (0.04) 6.3[d] (0.4)	0.18 (0.03) 9.3 (0.7)	0.18 (0.07) 9.1 (0.3)	0.19 (0.01) 9.6 (0.3)	0.11 (0.02) 8.1 (0.5)
Glutamic Acid	0.04 (0.01) 4.0 (0.8)	0.15 (0.06) 7.3 (1.6)	0.07 (0.04) 5.9 (2.1)	0.12 (0.09) 7.2 (4.3)	0.18 (0.06) 6.7 (1.5)	0.20 (0.10) 9.8[e] (2.9)	0.11 (0.03) 5.7 (1.1)	0.17 (0.11) 7.7 (3.6)	0.12 (0.01) 5.7 (0.3)	0.04 (0.01) 3.1 (0.1)
Glycine	0.07 (0.02) 6.7 (0.4)	0.11 (0.02) 5.5 (0.2)	0.08 (0.03) 7.0 (0.2)	0.11 (0.08) 6.4 (3.2)	0.15 (0.06) 5.5 (1.2)	0.11 (0.03) 5.4 (0.4)	0.13 (0.03) 6.7 (0.6)	0.13 (0.06) 6.4 (1.0)	0.12 (0.02) 5.9 (0.4)	0.07 (0.01) 5.1 (0.5)
Serine	0.06 (0.01) 5.4 (1.1)	0.13 (0.04) 6.3 (0.8)	0.07 (0.03) 5.8 (0.4)	0.11 (0.07) 6.4 (2.7)	0.17 (0.06) 6.0 (0.8)	0.11 (0.03) 5.4 (0.5)	0.12 (0.02) 6.2 (0.8)	0.15 (0.08) 7.2 (1.6)	0.12 (0.01) 6.1 (0.3)	0.06 (0.01) 4.4 (0.2)
Arginine	0.06 (0.01) 5.6 (1.8)	0.08 (0.02) 3.7 (0.1)	0.05 (0.01) 4.8 (1.6)	0.05 (0.01) 3.7 (1.5)	0.09 (0.02) 3.4 (1.5)	0.08 (0.02) 4.6 (2.3)	0.10 (0.05) 5.2 (1.3)	0.09 (0.03) 4.8 (0.5)	0.08 (0.02) 3.8 (0.6)	0.06 (0.02) 4.0 (0.9)
Glutamine	0.02 (0.01) 1.5 (0.3)	0.06 (0.01) 3.2 (0.2)	0.03 (0.02) 2.7 (0.8)	0.03 (0.02) 1.7 (0.1)	0.11 (0.03) 4.2 (0.1)	0.17 (0.06) 8.3[e] (0.6)	0.06 (0.01) 3.1 (1.0)	0.12 (0.08) 5.7 (2.6)	0.06 (0.01) 3.1 (0.3)	0.03 (0.01) 2.4 (1.1)
Isoleucine/ Leucine	0.11 (0.03) 10.2 (1.8)	0.24 (0.03) 12.3 (2.0)	0.13 (0.05) 10.8 (1.9)	0.10 (0.07) 5.6[d] (1.5)	0.35 (0.11) 12.7 (2.5)	0.17 (0.06) 8.6 (1.1)	0.24 (0.08) 11.9 (1.7)	0.21 (0.08) 9.9 (1.8)	0.28 (0.04) 14.0[e] (2.2)	0.17 (0.02) 12.1 (1.3)
Others[c]	0.01 (0.01) 1.2 (0.4)	0.04 (0.01) 2.2 (0.5)	0.02 (0.01) 1.9 (0.07)	0.02 (0.01) 0.3 (0.3)	0.07 (0.03) 2.5 (0.7)	0.04 (0.01) 1.7 (0.3)	0.04 (0.01) 1.9 (0.4)	0.05 (0.02) 2.4 (0.2)	0.06 (0.02) 3.1 (0.9)	0.03 (0.06) 2.1 (0.2)
Totals	1.04 (0.23)	2.00[e](0.38)	1.19 (0.38)	1.60 (0.72)	2.71[e](0.68)	2.02[e](0.57)	1.96 (0.40)	1.97[e](0.71)	2.00[e](0.14)	1.40 (0.11)

[a]concentration (nmol/mg wet weight) (S.D. in parentheses; n = 3)
[b]percent contribution to total FAA pool
[c]includes aspartic acid, asparagine, methionine and phenylalanine
[d]significantly decreased from control ($P \leq 0.05$)
[e]significantly increased from control ($P \geq 0.05$)

Table 6. Concentration (nmol/mg wet weight) and percent composition of FAA in adductor muscles of *E. complanata* after *in situ* exposure for 77-79 d.

Amine Acid	35	4	5	32	33	37
Alanine	0.44[a] (0.07) 23.5[b] (0.5)	0.59[e] (0.12) 17.7[d] (0.4)	0.25 (0.08) 22.9 (0.6)	0.60 (0.12) 19.3 (2.5)	0.45 (0.10) 19.5 (1.8)	0.39 (0.09) 24.0 (0.9)
Proline	0.44 (0.02) 23.8 (3.0)	0.74 (0.12) 22.6 (3.9)	0.24 (0.08) 21.9 (0.6)	0.66 (0.21) 21.5 (7.4)	0.45 (0.15) 19.1 (1.0)	0.25 (0.07) 15.4 (3.8)
Valine	0.17 (0.05) 9.3 (1.1)	0.27 (0.03) 8.1 (1.0)	0.12 (0.02) 11.2 (2.1)	0.27 (0.02) 8.7 (0.5)	0.16 (0.03) 7.0 (0.9)	0.16 (0.01) 9.6 (1.3)
Threonine	0.19 (0.02) 10.3 (0.8)	0.33 (0.09) 9.8 (0.9)	0.10 (0.03) 9.0 (0.3)	0.31 (0.03) 10.2 (1.5)	0.22 (0.09) 9.3 (1.5)	0.14 (0.04) 8.5 (1.0)
Glutamic Acid	0.10 (0.02) 5.1 (0.7)	0.22 (0.07) 6.7 (1.4)	0.05 (0.04) 4.4 (2.0)	0.24 (0.05) 7.8 (1.2)	0.20 (0.08) 8.5 (1.2)	0.10 (0.06) 5.5 (2.4)
Glycine	0.14 (0.02) 7.7 (0.8)	0.27 (0.07) 8.1 (0.4)	0.08 (0.04) 7.0 (0.6)	0.26 (0.06) 8.2 (1.7)	0.19 (0.01) 7.9 (0.4)	0.12 (0.05) 7.2 (1.7)
Serine	0.12 (0.03) 6.3 (0.8)	0.25 (0.06) 7.5 (0.6)	0.07 (0.02) 6.3 (0.2)	0.21 (0.05) 6.9 (1.6)	0.17 (0.04) 7.2 (0.2)	0.11 (0.04) 6.3 (1.2)
Arginine	0.04 (0.03) 2.1 (1.3)	0.12 (0.03) 3.7 (0.2)	0.05 (0.02) 5.0[e] (0.9)	0.12 (0.02) 3.8 (0.9)	0.10 (0.02) 4.2 (0.6)	0.05 (0.01) 3.1 (0.2)
Glutamine	0.04 (0.02) 2.0 (0.9)	0.13 (0.04) 3.8 (0.6)	0.02 (0.01) 1.5 (0.6)	0.08 (0.02) 2.7 (0.4)	0.12 (0.06) 4.8 (1.0)	0.06 (0.01) 3.8 (0.2)
Isoleucine-Leucine	0.16 (0.06) 8.3 (2.3)	0.32 (0.07) 9.8 (0.9)	0.10 (0.03) 9.1 (2.2)	0.29 (0.10) 9.2 (2.8)	0.22 (0.03) 9.7 (1.5)	0.21 (0.04) 13.3[e] (3.6)
Others	0.03 (0.02) 1.6 (0.9)	0.07 (0.02) 2.2 (0.5)	0.02 (0.01) 1.7 (0.5)	0.06 (0.03) 1.8 (1.0)	0.06 (0.02) 2.8 (1.0)	0.05 (0.02) 3.4[e] (1.7)
Totals	1.88 (0.29)	3.32[e] (0.66)	1.08[d] (0.37)	3.09[e] (0.18)	2.32 (0.64)	1.64 (0.33)

[a]concentration (nmol/mg wet weight) (S.D. in parentheses; n = 3)
[b]percent contribution to total FAA pool
[c]includes aspartic acid, asparagine, methionine and phenylalanine
[d]significantly decreased from control ($P \leq 0.05$)
[e]significantly increased from control ($P \geq 0.05$)

Table 7. Concentration (nmol/mg wet weight) and percent composition of FAA in adductor muscles of E. complanata after in situ exposure for 27 .9 d.

Amine Acid	35 (Control)	4	5	8	30	31	32	33	34	37
Alanine	0.19 (0.04) 16.1 (0.3)	0.35 (0.0?) 18.7 (0.9)	0.21 (0.06) 18.6 (1.3)	0.20 (0.10) 19.8 (2.5)	0.39 (0.11) 17.1 (1.0)	0.30 (0.03) 16.0 (1.5)	0.28 (0.08) 14.6 (1.0)	0.28 (0.03) 16.1 (1.2)	0.29 (0.05) 15.4 (1.3)	0.29 (0.09) 17.4 (0.1)
Glutamic Acid	0.13 (0.02) 10.8 (1.1)	0.24 (0.05) 12.7 (1.2)	0.15 (0.06) 13.4 (2.6)	0.11 (0.07) 10.2 (3.3)	0.28 (0.10) 11.9 (1.8)	0.31 (0.10) 16.6[e] (4.3)	0.23 (0.04) 12.4 (1.2)	0.24 (0.04) 13.9 (1.5)	0.21 (0.06) 10.6 (1.9)	0.19 (0.08) 11.4 (3.0)
Threonine	0.12 (0.03) 9.8 (0.9)	0.19 (0.05) 10.1 (1.0)	0.12 (0.03) 10.5 (0.8)	0.11 (0.04) 11.4 (1.7)	0.22 (0.05) 9.5 (0.4)	0.13 (0.03) 6.8[d] (1.2)	0.19 (0.04) 10.0 (0.3)	0.15 (0.01) 8.8 (0.2)	0.18 (0.02) 9.3 (0.2)	0.16 (0.04) 9.5 (0.4)
Glycine	0.11 (0.02) 9.0 (1.6)	0.17 (0.02) 9.4 (1.0)	0.12 (0.05) 10.9 (1.3)	0.11 (0.05) 10.3 (1.1)	0.19 (0.05) 8.3 (0.4)	0.13 (0.02) 7.2 (0.9)	0.16 (0.03) 8.6 (0.5)	0.18 (0.02) 10.6 (0.6)	0.14 (0.01) 7.4 (0.8)	0.14 (0.05) 8.4 (0.6)
Serine	0.10 (0.01) 7.9 (1.0)	0.18 (0.02) 9.4 (0.8)	0.11 (0.05) 9.6 (1.5)	0.11 (0.05) 11.0 (3.2)	0.17 (0.04) 7.4 (0.3)	0.11 (0.02) 5.8 (0.7)	0.13 (0.03) 7.0 (0.5)	0.14 (0.02) 7.8 (0.5)	0.15 (0.03) 7.5 (1.2)	0.12 (0.03) 7.2 (0.5)
Proline	0.12 (0.05) 10.0 (2.5)	0.15 (0.03) 7.8 (0.1)	0.08 (0.03) 7.3 (0.3)	0.10 (0.05) 10.0 (2.3)	0.19 (0.02) 8.7 (1.6)	0.13 (0.02) 6.8 (0.4)	0.14 (0.04) 7.1 (0.3)	0.12 (0.01) 6.8[d] (0.3)	0.15 (0.03) 7.7 (1.7)	0.14 (0.05) 8.2 (0.9)
Valine	0.12 (0.04) 10.0 (1.9)	0.17 (0.04) 8.8 (0.5)	0.09 (0.03) 8.4 (0.3)	0.10 (0.05) 10.4 (2.8)	0.21 (0.05) 9.3 (0.4)	0.12 (0.02) 6.5 (0.8)	0.16 (0.04) 8.2 (0.5)	0.11 (0.01) 6.6 (0.6)	0.17 (0.03) 8.5 (2.3)	0.14 (0.05) 8.7 (1.5)
Glutamine	0.06 (0.01) 5.2 (0.9)	0.10 (0.03) 5.1 (0.6)	0.06 (0.01) 5.6 (0.4)	0.04 (0.02) 3.6 (1.3)	0.12 (0.04) 5.3 (0.7)	0.25 (0.07) 13.3[e] (3.7)	0.08 (0.02) 4.1 (1.0)	0.10 (0.02) 5.5 (0.7)	0.09 (0.01) 4.5 (0.5)	0.09 (0.04) 5.4 (0.5)
Arginine	0.06 (0.01) 5.1 (1.9)	0.06 (0.01) 3.4 (1.0)	0.05 (0.02) 4.7 (2.4)	0.07 (0.02) 7.0 (1.2)	0.13 (0.03) 6.0 (2.7)	0.06 (0.03) 2.9 (1.4)	0.14 (0.05) 7.4 (0.9)	0.08 (0.01) 4.6 (0.9)	0.10 (0.03) 5.3 (1.2)	0.08 (0.03) 4.7 (1.2)
Isoleucine/ Leucine	0.13 (0.03) 11.2 (1.5)	0.23 (0.06) 12.1 (2.5)	0.10 (0.04) 9.2 (1.9)	0.05 (0.05) 4.8 (2.1)	0.30 (0.12) 12.8 (3.3)	0.20 (0.07) 10.8 (4.0)	0.25 (0.07) 13.3 (0.6)	0.24 (0.03) 13.9 (1.7)	0.23 (0.05) 12.2 (2.7)	0.24 (0.09) 14.2 (2.9)
Others[c]	0.06 (0.03) 4.8 (1.7)	0.05 (0.02) 2.7 (0.9)	0.02 (0.01) 1.8 (0.7)	0.02 (0.03) 1.4 (2.0)	0.08 (0.03) 3.7 (0.8)	0.05 (0.01) 2.7 (0.6)	0.06 (0.01) 3.2 (0.7)	0.04 (0.01) 2.1 (0.7)	0.09 (0.02) 4.2 (1.3)	0.05 (0.02) 3.1 (1.0)
Totals	1.20 (0.22)	1.88 (0.38)	1.11 (0.35)	1.02 (0.51)	2.29[e] (0.56)	1.85 (0.13)	1.88 (0.43)	1.72 (0.14)	1.85 (0.25)	1.66 (0.51)

[a] concentration (nmol/mg wet weight) (S.D. in parentheses; n = 3)
[b] percent contribution to total FAA pool
[c] includes aspartic acid, asparagine, methionine and phenylalanine
[d] significantly decreased from control ($P \leq 0.05$)
[e] significantly increased from control ($P \geq 0.05$)

Table 8. Concentration (nmol/mg wet weight) and percent composition of FAA in adductor muscles of *E. complanata* after *in situ* exposure for 77-79 d.

Amine Acid	Control	4	5	32	33	37
Alanine	0.29[a] (0.04) 19.7[b] (0.6)	0.34 (0.06) 17.3 (2.8)	0.20 (0.04) 17.6 (2.2)	0.83 (0.25) 18.7 (1.8)	0.46 (0.16) 16.0 (0.4)	0.39 (0.08) 17.2 (1.2)
Glutamic Acid	0.20 (0.04) 10.5 (3.8)	0.25 (0.02) 13.5 (0.3)	0.13 (0.03) 11.0 (2.1)	0.60 (0.22) 11.5 (2.8)	0.31 (0.06) 11.1 (2.3)	0.30 (0.06) 13.3 (1.6)
Threonine	0.15 (0.01) 9.4 (0.5)	0.17 (0.02) 9.5 (0.8)	0.12 (0.01) 10.3 (0.6)	0.42 (0.07) 9.6 (1.4)	0.33 (0.09) 7.8[d] (0.6)	0.21 (0.02) 9.2 (0.6)
Glycine	0.16 (0.04) 9.7 (0.4)	0.22 (0.01) 11.4 (0.4)	0.10 (0.03) 8.4 (1.5)	0.45 (0.12) 9.9 (0.6)	0.29 (0.13) 9.7 (0.9)	0.20 (0.03) 9.1 (0.5)
Serine	0.14 (0.03) 8.1 (0.6)	0.16 (0.01) 8.8 (1.2)	0.09 (0.02) 7.9 (0.6)	0.33 (0.11) 7.7 (0.3)	0.22 (0.09) 7.6 (0.7)	0.18 (0.02) 7.8 (0.7)
Proline	0.13 (0.01) 8.1 (0.6)	0.16 (0.02) 8.1 (0.6)	0.10 (0.02) 8.5 (0.7)	0.38 (0.08) 8.6 (1.0)	0.18 (0.08) 6.2 (0.5)	0.16 (0.02) 7.1 (0.1)
Valine	0.17 (0.03) 8.6 (1.5)	0.13 (0.02) 7.2 (0.7)	0.11 (0.03) 7.5 (1.3)	0.33 (0.06) 8.8 (2.1)	0.17 (0.07) 5.7[d] (0.2)	0.18 (0.01) 8.1 (0.8)
Glutamine	0.07 (0.01) 4.2 (1.1)	0.08 (0.01) 4.2 (0.2)	0.05 (0.01) 4.1 (0.6)	0.17 (0.05) 3.9 (0.3)	0.16 (0.08) 5.1 (0.8)	0.11 (0.01) 4.9 (0.8)
Arginine	0.09 (0.05) 4.8 (3.3)	0.11 (0.07) 3.3 (1.2)	0.10 (0.02) 9.2[e] (2.7)	0.26 (0.11) 5.8 (2.4)	0.23 (0.07) 8.4[e] (2.8)	0.07 (0.01) 3.1 (0.8)
Isoleucine-Leucine	0.15 (0.03) 10.8 (1.4)	0.21 (0.04) 9.5 (3.6)	0.10 (0.01) 8.7 (1.4)	0.61 (0.39) 13.3 (4.0)	0.53 (0.25) 17.9 (2.0)	0.39 (0.16) 17.1 (6.4)
Others[c]	0.05 (0.02) 2.7 (0.3)	0.05 (0.01) 2.8 (0.4)	0.03 (0.02) 2.9 (1.9)	0.07 (0.02) 1.9 (0.5)	0.07 (0.03) 2.3 (0.3)	0.07 (0.01) 3.2 (0.7)
Totals	1.59 (0.18)	2.04 (0.21)	1.13 (0.11)	4.39[e] (1.50)	2.92 (1.06)	2.26 (0.29)

[a] concentration (nmol/mg wet weight) (S.D. in parentheses; n = 3)
[b] percent contribution to total FAA pool
[c] includes aspartic acid, asparagine, methionine and phenylalanine
[d] significantly decreased from control ($P \leq 0.05$)
[e] significantly increased from control ($P \geq 0.05$)

Table 9. Condition Index (C.I.) of *E. complanata* after 27-29 and 77-79 d *in situ* at each study site.

Site	Exposure Time (d) 27-29	77-79
35	1.8 (0.1)	2.1 (0.5)
4	2.2 (0.1)	2.0 (0.1)
5	1.9 (0.3)	1.5 (0.1)
8	1.9 (0.3)	1.2[a] (0.2)
30	2.3 (0.3)	---
31	2.3 (0.3)	---
32	2.0 (0.3)	2.1 (0.2)
33	2.4 (0.1)	2.0 (0.2)
34	2.3 (0.2)	---
37	2.3 (0.2)	2.1 (0.2)

[a]significantly different from control site #35

Table 10. Comparison of Sites Using Hilsenhoff Biotic Index

Station	Benthic Invertebrate Sample	Index (0-10)	Rating
Control	artificial	-[a]	-[a]
	surber	4.9	good
4	artificial	6.5	fairly poor
	surber	-[a]	-[a]
5	artificial	8.3	very poor
	surber	5.8	fairly poor
8	artificial	5.9	fairly poor
	surber	4.3	good
37	artificial	5.7	fairly poor
	surber	3.9	very good
30	artificial	9.9	very poor
	surber	-[a]	-[a]
31	artificial	7.2	poor
	surber	5.9	fairly poor
32	artificial	7.2	poor
	surber	5.5	fair
33	artificial	8.6	very poor
	surber	-[a]	-[a]
34	artificial	-[a]	-[a]
	surber		

[a]no sample taken

Table 11. r^2 values for linear correlations between total FAA and measured environmental variables.

Environmental Parameter	Adductor	Mantle	Gill
Depth	0.02	0.07	0.01
Velocity	0.06	0.12	0.44
Temperature	0.05	0.13	0.22
D.O.	<0.01	0.53	0.07
pH	0.02	0.50	<0.01
Alkalinity	0.06	0.23	0.16
Susp. Solids	0.01	0.13	0.12
Conductivity	0.14	0.45	0.32
NH_4N	0.11	0.08	0.12
NO_3NO_2	0.12	<0.01	0.10
Phosphorus	0.39	0.12	0.24
POC	<0.01	0.49	0.32
DOC	0.03	0.10	0.08
DIC	0.04	0.12	0.09
Al	0.02	0.40	0.11
Fe	<0.01	0.47	0.38
Zn	0.10	0.01	0.01

Table 12. Summary of changes in total FAA concentrations in three tissues.

Tissue	27-29 d	77-79 d
Adductor muscles	4* , 33* 5 , 30 , 31 , 34 , 37	4* , 33* , 37* 32* 5 No data available for 30, 31, 34
Mantle	4* , 30* , 31* , 33* , 34* 8 , 32 , 37	4* , 32* 5* 33 , 37 No data available for 30, 31, 34
Gill	30*	32*

*Statistically significant ($P \leq 0.05$)

Table 13. Ranking of study sites based on FAA changes and the Hilsenhoff biotic index.

	FAA	B.I.*
Worst	4,33	30,33
	30,31,34	5
	5	31,32
	32	4
	37	8
	8	37
Best	35	35

*site #34 not assessed

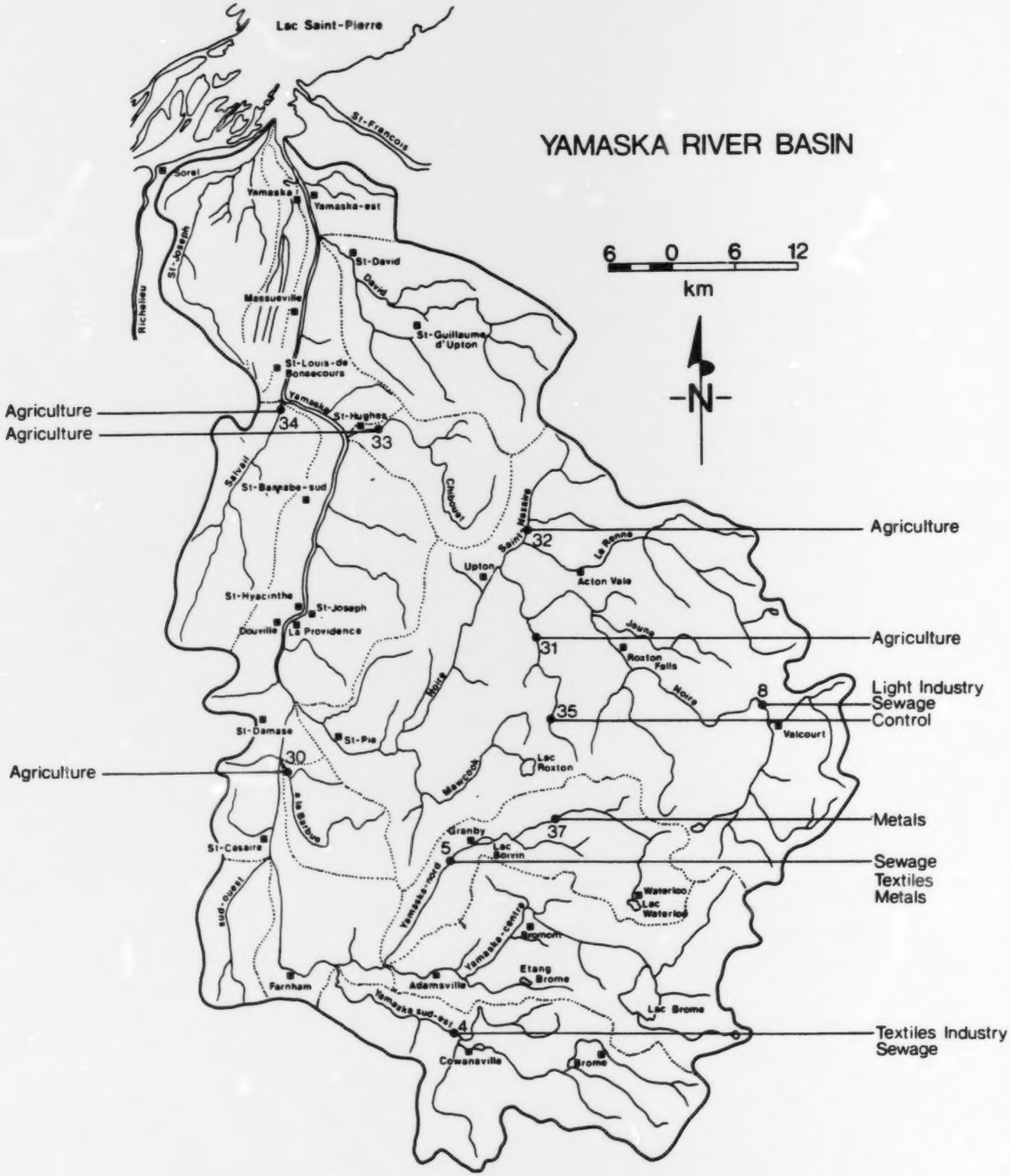

Figure 1. Study sites for caged mussels and benthic invertebrate samples.